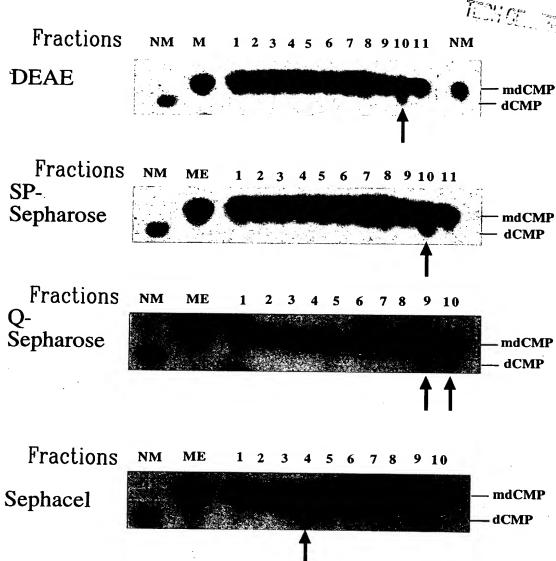


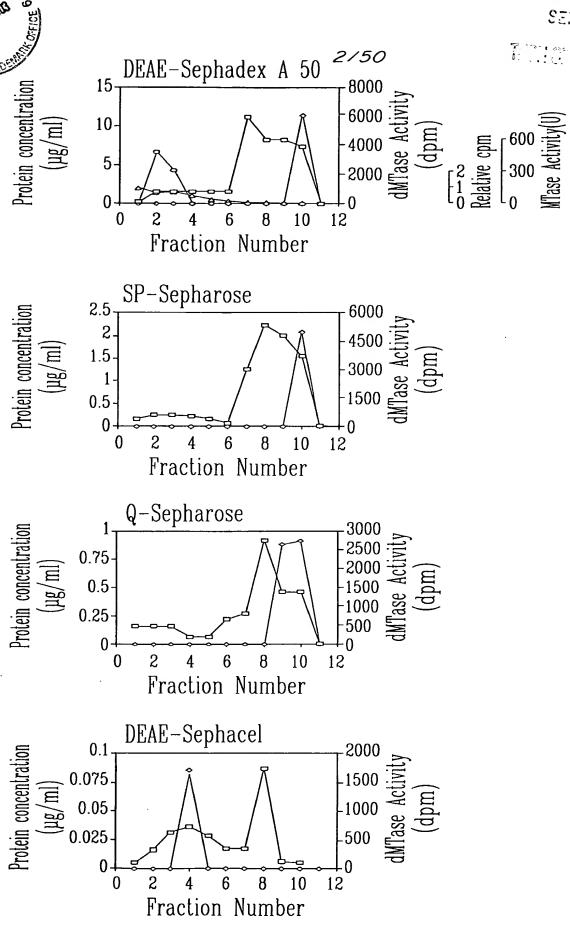
SEP 2 9 2603



pmCpGpmCpG MNase mCp + Gp

TI IA

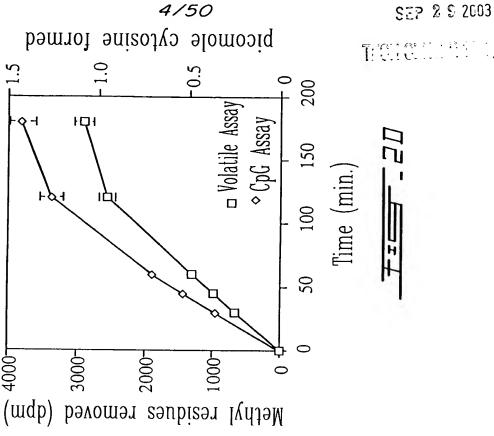
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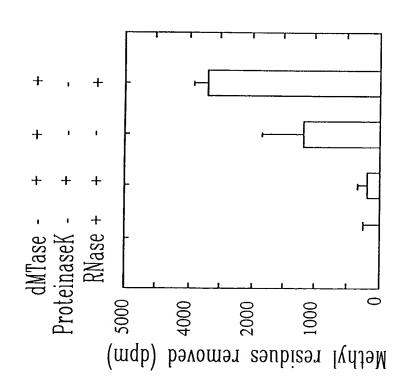


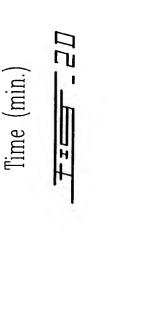
THALE	3/50		SEP 2 9 2003
	,		
######################################	— mdCMP — dCMP	<u> </u>	- mdCMP
Proteinase K		7 <del>4</del> P	
-RNase	*	ч ε	
mdCTP		11.77	
qqCLP		7 Р	
Sephacel		ЧІ	
S-Sepharose Q-Sepharose		nim 24	
DEAE-Sephadex		nim 0£	
ME	•	ME	
WN	•	WN	

Serial No. 09/554,414 REPLACEMENT SHEET

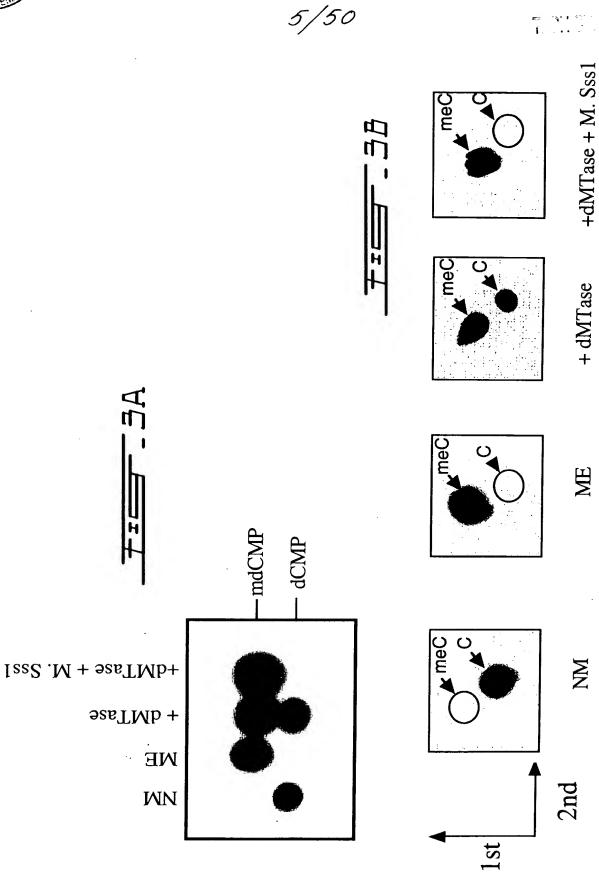


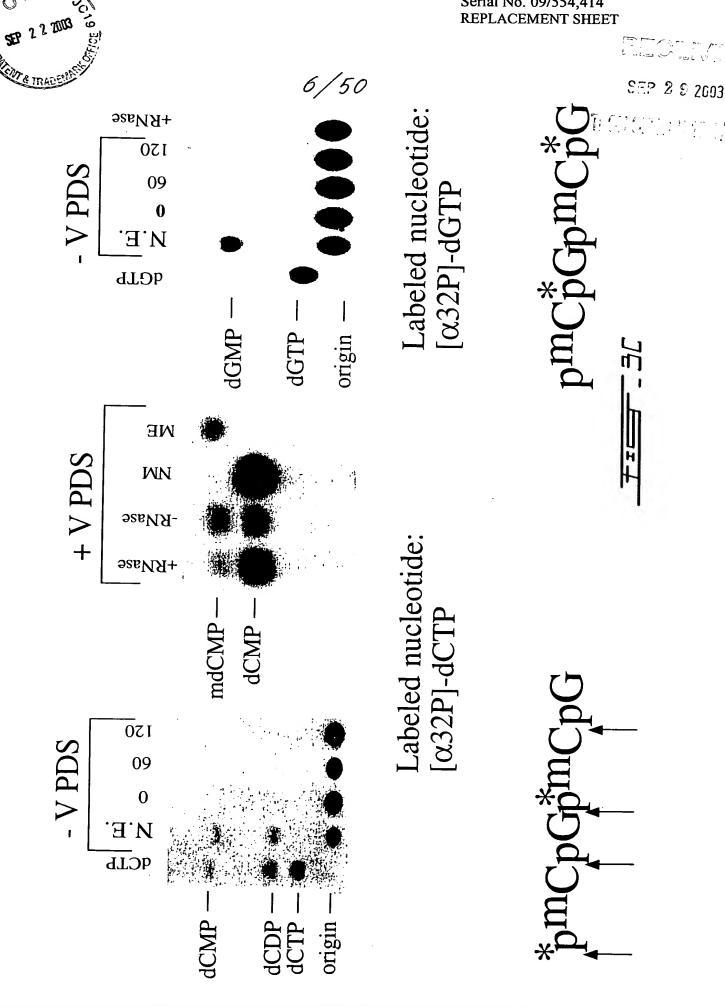


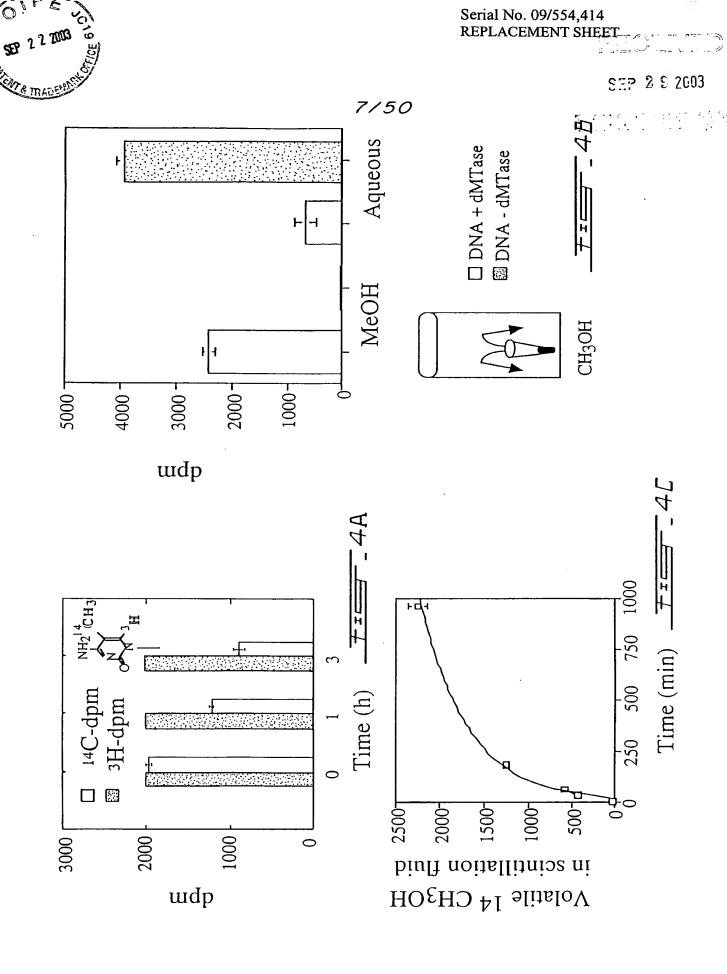












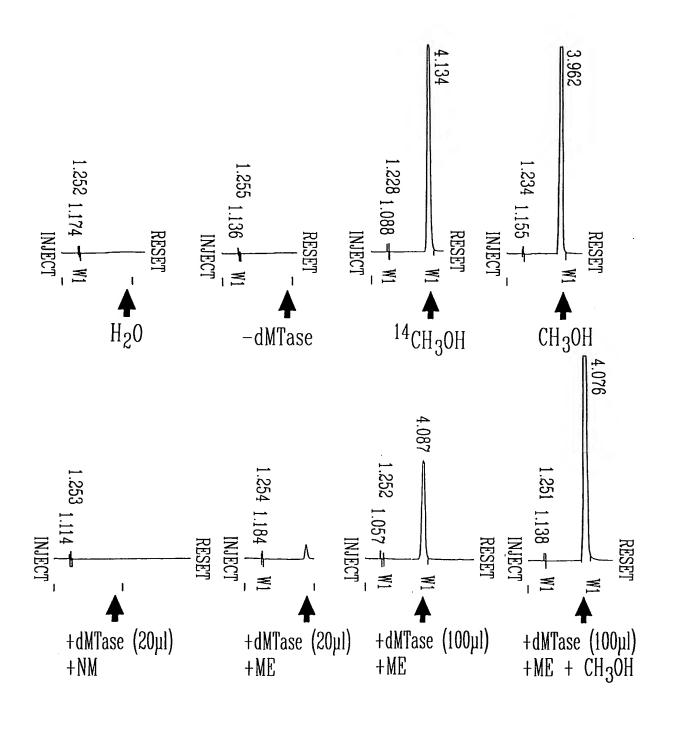
SEP 2 S 2003



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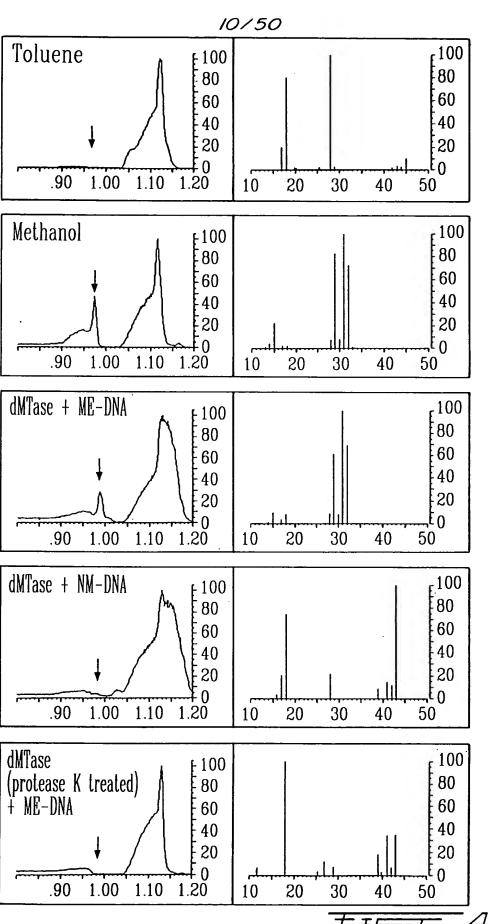


SE? 2 9 2003



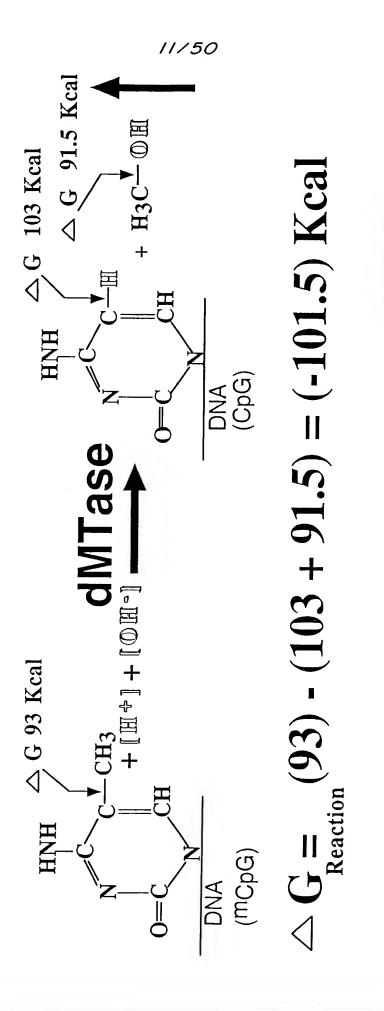
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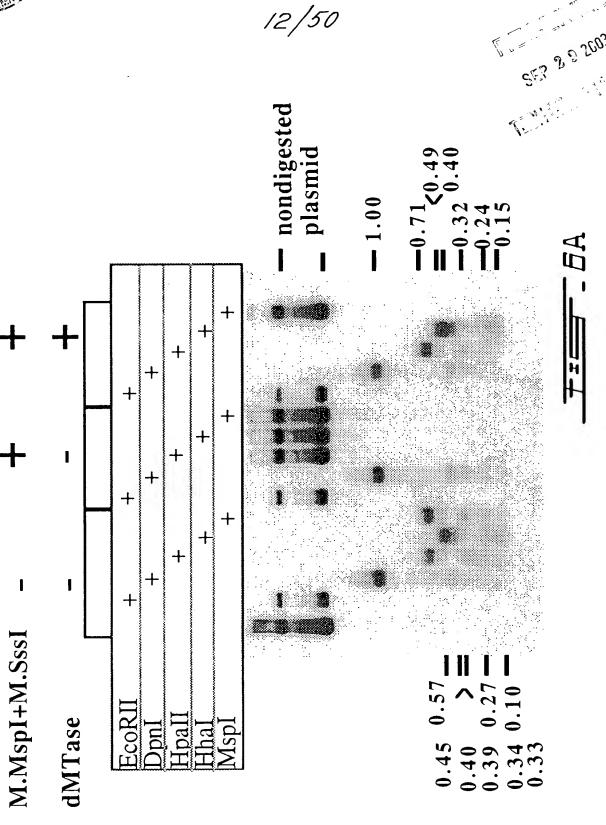
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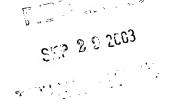


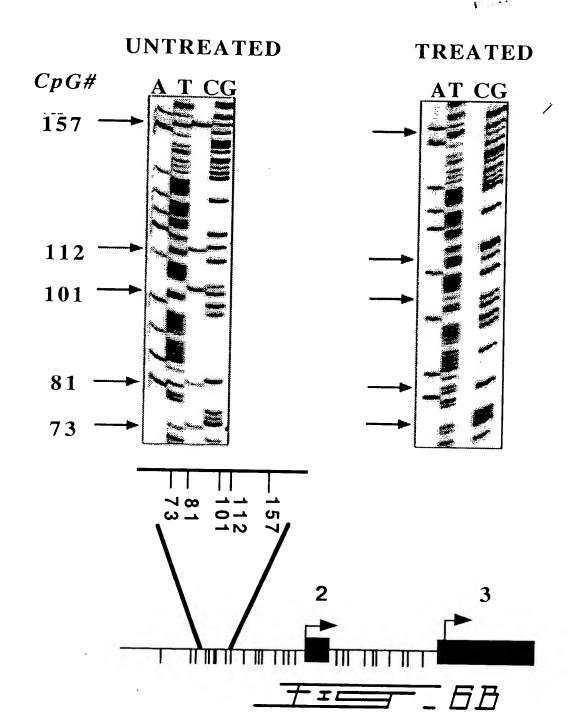
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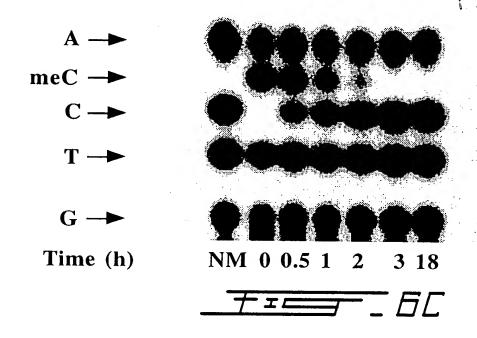


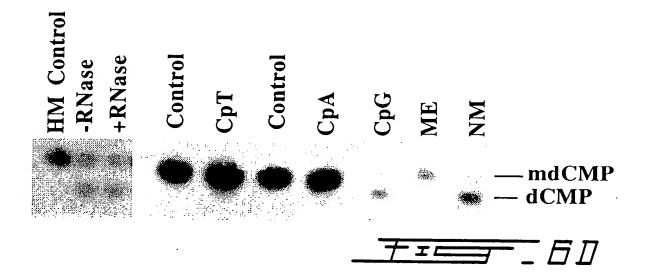


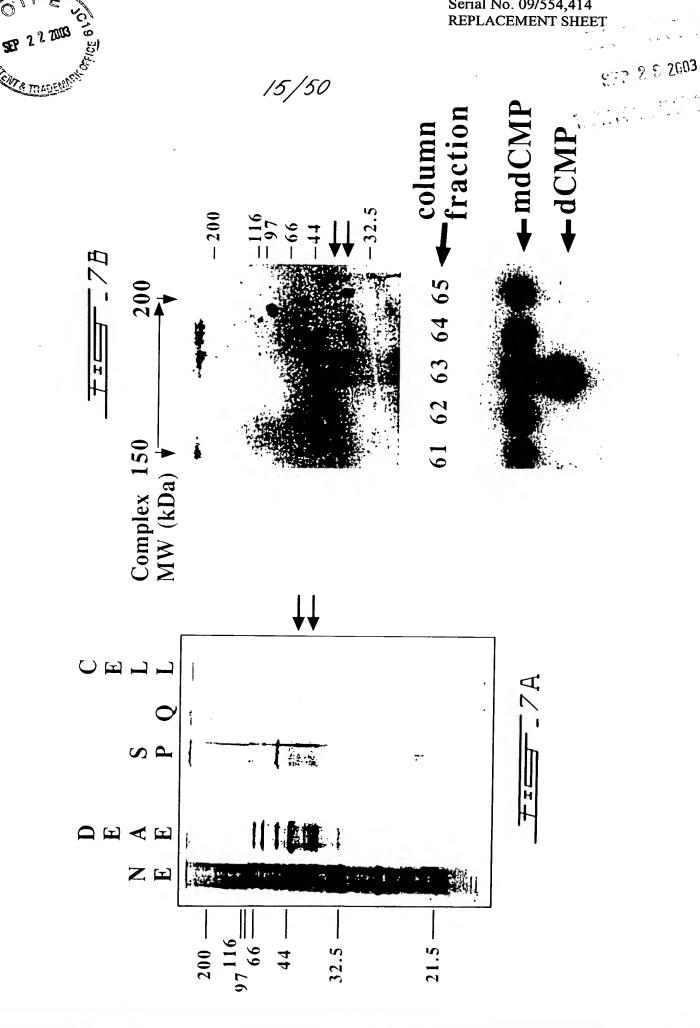




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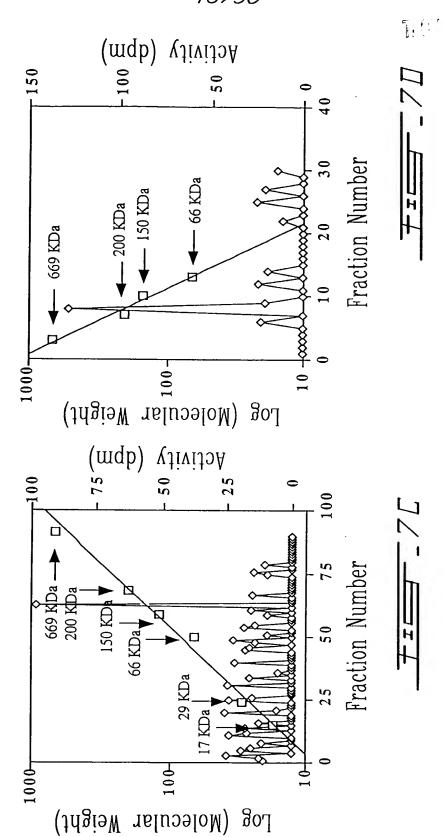








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SP 22 700 SS

DCPAL,PPGMKKEEN

EST

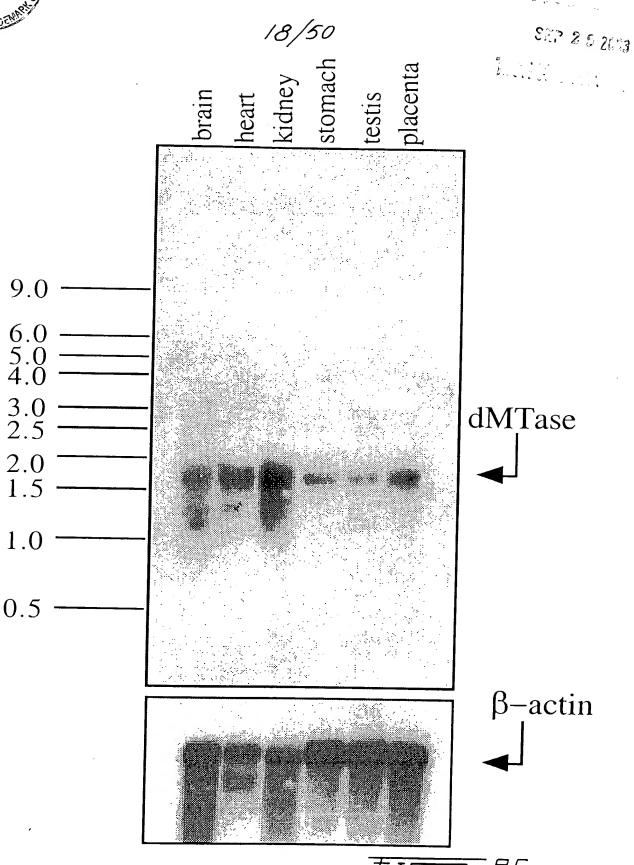
17/50

SEP 2 9 2003

57 - 21 - 22 - 2003

<i>T===-</i> BA		homology to methylated DNA	binding domain	homology to coiled	con domain		T=====================================
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	KSDVYY FSPSGKKFRS	MPSKLQ KNKQRLRNDE	SIFKQPVTKV TNHPSNKVKS	LSASDVTEQI IKTMELPKGL	APITGQ VSAAVEKNPA	ERVOOV RKILEDALMA	
	MDCPALPPGW KKEEVIRKSG LSAGKSDVYY FSPSGKKFRS 40	KPOLARYLGN TVDLSSFDFR TGKMMPSKLQ KNKQRLRNDP SZSZZZZZZZZZZZZZZZ	LNQNKGKPDL NTTLPIRQTA SIFK	DPQRMNEQPR QLFWEKRLQG LSAS	QGVGPGSNDE TLLSAVASAL HTSSAPITGQ VSAAVEKNPA	VWLNTSQPLC KAFIVTDEDI RKQEERVOOV RKILEDALMA 240	DILSRAADTE EMDIEMDSGD EA





SEP 2 5 703

19/50

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Human DNA demethylase cDNA-dMTase1 and predicted amino acid sequence

+x==== 91



tttagatgta aaaccatgga tggatattga gactttccc tgtaagaaaa ttttctggga ttttatctgc acgatgatgt cttttattgt agaaattgga taggacttaa tgatgccagg agttttaata attattgtga cttcactgta tacattcttc catcatcact ccacgtcagc gggcaagtct ttttgagtcc tttcccccac actttcgacc ggcttttgcc ccaatgcctt gctgagcttt caaattataa gatgagaccc ctctgcaaag caagtacgca acagaagaga aaaaaa gaatgaacag gcgagtacag tgatcaggta aaaacatttg gccaatcaca atctcaaccc tgtttccact atagcactaa gggacacctg tgtaacagaa agctgctgat atcatgttta aggtagcaat ctaagatgaa ccagttaaag aaaaaaaa cacaacgaat caagctctgc cctaagaata gtgcatcaga gagttggtcc ggcttaacac aacaggaaga gagcttttta aagcaggacc aataaatttc ttattgaaag tgaacaaaaa gcacttacgt atattcacaa tcttgtcgcg tattcaaaaa aaatcagacc caaggactta ggtcttcaag gctttgcaca cctgctgttt atggcagaca ggagatgaag tcctagaaat cgagcacata tggaaatgta ttttatact ataaatgatt ttgtaatatc aggaattcta gacatcagga atatatctat ggaaaagaac taataaagtg gaagaggcta actacccaaa tgttgccagt cacagatgaa agaagcactg aatggacagt aagrgaaat aaaatgtacc tttttgatgt aattaqtctt tgcaatctac agaacagatc tataaacata gtaaagacaa

SEQ ID NO:1



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DCPALPPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGNT

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WLNTSQPLCKAFIVTDEDIRKQEERVQQVRKKLEEALMADILSRAADTEE

MDIEMDSGDEA

I FKQPVTKVTNHPSNKVKSDPQRMNEQPRQLFWEKRLQGLSASDVTEQII



SEQ ID NO:2



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22/50

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Human DNA demethylase homologue-dMTase2 and predicted amino acid sequence

SEP 2 0 2003

23/50

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SEQ ID NO:3

SE? 2 8 2003

24/50

SEQ ID NO:4 75

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\_ <u>96</u> .

Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12 Lipman-Pearson Protein Alignment

							;	25/	50
Consensus	Length	250	250		TGKMMPSK	TGKM: SK	TGKMI MSK,	0.	
Gap	Length	0	0	v210	TVDLSSFDFR	::DIS:FDFR	SMDLSTFDFR	09~	
Gap	Number	0	0	v200	KFQLARYLGN	KPQLARYLG.	KPQLARYLGG	~20	
Similarity	Index	76.0	76.0	v190	SPSCKKFRS	SPSCKKFRS	SPSCKKFRS	~40	
Seq1(1>411) Seq2(1>291) Seq2(1>291)	human dMTase2 protein	(4>253)	(4>253)	v170 v180	KRMDCPALPPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGNIVDLSSFDFRIGKMPSK	KR : CPALP.GW.: EEV R: SGLSAG DV: Y: SPSGKKFRSKPQLARYLG.:: DLS: FDFRIGKM: SK	KRWECPALPOGWEREEVPRRSGLSAGHRDVFYYSPSGKKFRSKPQLARYLGGSMDLSTFDFRTGKMLMSK	~30	
Se		4)		v160 v	PPGMKKEEVIR	P.GW.: EEV R.	POGMEREEVPRI	.0 ^20	
Seq1(1>411)	human dMTase1 protein	(148>397)	(148>397)	v150	KRMDCPAL	KR:CPAL	KRWECPAL	^10	

^140 MIKSRQRVRYDSSNQVKGKPDLNTAL.PVRQTASIFKQPVIKITINHPSNKVKSDPQKAVDQPRQL.FWEKKL : OPROLEWEK: L LOKNIKORLIRNDPLINONIKGKPDLINTTT.PIROTASIFKOPVIKVIINHPSNIKVKSDPORMNEOPROLFWEKRL **v**280 ^130 ::K::QR:R D: NQ KGKPDLNT:LP:RQTASIFKQFVTK:TNHPSNKVKSDPQ: V270 ^120 v260 $^{110}$ v250^100 99 \ 08√

v240

v230

v220



u 13,22, C

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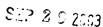
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v360 v370 v380 v390 LCKAFIVIDEDIRKQEERVQQVRKKI EEALMADII SRAAD LCKAF:VIDEDIRKQEE VQQVRK: LEEALMAD: L.:..: LCKAFMVIDEDIRKQEELVQQVRKRI EEALMADMLAHVEE

^220 ^230

^240

^2





## Mouse DNA demethylase-dMTase1 and predicted amino acid sequence

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SEQ ID NO:5



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KRMDCPALPPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYL

GNAVDLSSFDFRTGKMMPSKLQKNKQRLRNDPLNQNKGKPDLNTTLPIRQ

TASIFKQPVTKFTNHPSNKVKSDPQRMNEQPRQLFWEKRLQGLSASDVTE

**QIIKTMELPKGLQGVGPGSNDETLLSAVASALHTSSAPITGQVSAAVEKN** 

PAVWLNTSQPLCKAFIVTDEDIRKQEERVQQVRKKLEEALMADILSRAAD

TEEVDIDMDSGDEA

SEQ ID NO:6

XE \_\_\_\_\_\_\_



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\$30 25 2503



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ID NO:7

SEQ

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NB -





RYLGGSMDLSTFDFRTGKMLMNKMNKSRQRVRYDSSNQVKGKPDLNTALP MERKRWECPALPQGWEREEVPRRSGLSAGHRDVFYYSPSGKKFRSKPQLA VRQTASIFKQPVTKITNHPSNKVKSDPQKAVDQPRQLFWEKKLSGLSAFD IAEELVRTMDLPKGLQGVGPGCTDETLLSAIASALHTSTLPITGQLSAAV EKNPGVWLNTAQPLCKAFMVTDDDIRKQEELVQQVRKRLEEALMADMLAH ID NO:8 SEQ VEELARDGEAPLDKACAEEEEEEEEEEEEEFFFFV

SEP 8020;

STE VO TO TO THE THE PARTY OF T

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty:

Niupie: 4; Gap Penaity: 4; Gap Length Penaity: 12	cap rength	Penalty: 12					
Seq1(1>414)	Seq2(1>285)		Similarity	Gap		Gap	Consensus
mouse dMTase1 protein	mouse dMTase2 protein	ase2 protein	Index	Number	Le	Length	Length
(151>400)	(4>253)		75.2	0		0	250
(151>400)	(4>253)		75.2	0		0	250
v160 v	v170 v	v180	v190	v200	v210	V220	
KRMDCPAL PPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGNAVDLSSFDFRIGKMPSK	TRKSGLSAC	<b>EKSDVYYFS</b>	SPSGKKFRS	KRQLARYI	GNAVDL	SSFDFRIC	KMMPSK
KR : CPALP.GW.: EEV R:SGLSAG DV:Y:SPSGKKFRSKPQLARYLG.::DLS:FDFRTGKM: :K	7 R:SGISAC	3DV:Y:S	SPSGKKFRS	KPQLARYI	.G.::DL	S: FDFRIC	KM: :K
KRWECPALPQGWEREEVPRRSGLSAGHRDVFYYSPSGKKFRSKPQLARYLGGSMDLSTFDFRTGKMLMIK	PRRSGLSAC	HRIVEYYS	SPSGKKFRS	KPQLARYI	GGSMDL	STEDFRIC	KMIMIK
^10	^20	√30	~40	<sup>^</sup> 50		09√	~70

v290 ^140 MNKSRQRVRYDSSNQVKGKPDLNTALPVRQTASIFKQPVIKTINHPSNKVKSDPQKAVDQPRQLFWEKKL LOKNKORLANDPI NONKGKPDI NITILPIRQIASIFKOPVIKFIINHPSNKVKSDPORMNEQPROLFWEKRL ::K::QR:R D: NQ KGKPDINT:LP:RQTASIFKQPVTK:TNHPSNKVKSDPQ: :QPRQLFWEK:L v280 v270 ^120 v260^110 v250 $^{100}$ v240<u>م</u> v230**08**√

15 - Est

SE 22 MB SE

34/50

v360 QELSASDVIEQIIKIMELEKGLQGVGEGSNDETLLSAVASALHTSSAPITGQVSAAVEKNPAVMLNTSQP ^210 SGLSAFDIAEELVRIMDLPKGLQGVGPGCIDEITLSAIASALHTSIT.PIIGQLSAAVEKNPGVMLNIAQP GLSA D::E::::TM:LPKGLQGVGPG..DETLLSA:ASALHTS: PITGQ:SAAVEKNP:VMLNT:QP v350  $^{\sim}200$ v340^190 v330 ^180 v320 ^170 v310 ^160  $v_{300}$ ^150

v370 v380 v390 v4

LCKAFIVIDEDIRKQEERVQQVRKKLEEALMADILSRAAD LCKAF:VID:DIRKQEE VQQVRK:LEEALMAD:L::..:

LCKAFWVIDDDIRKQEELVQQVRKRLEEALMADMLAHVEE

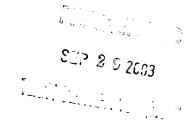
-KAFIMVIIDDILKKŲEIELVQQVKKRLIEIFALMALMLAHVETE ^220 ^230 ^240 ^250 FIET BP

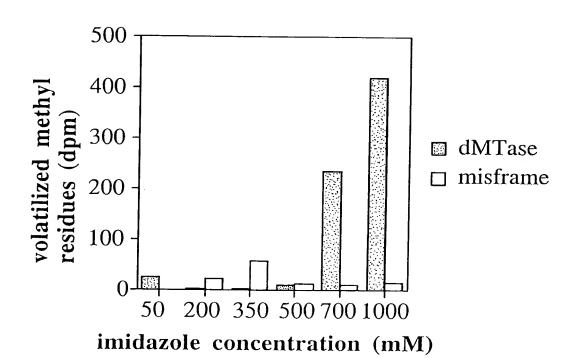
E,



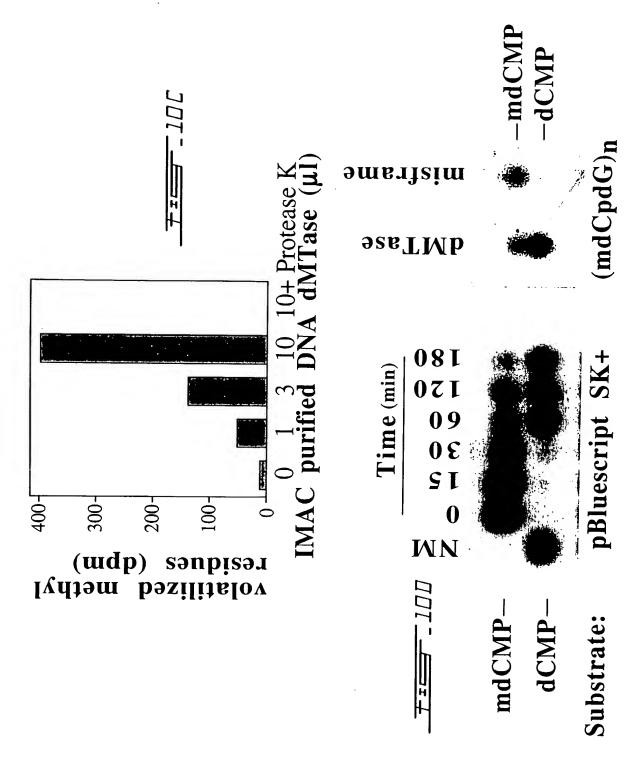
35/50 Not 1 T7 ATG (His) & XpressEK MCS BGHpA 1.36 kb 66 45 29 pcDNA-His-dMTase misframe 928 aga MDBD Not 1 **PCMV** 





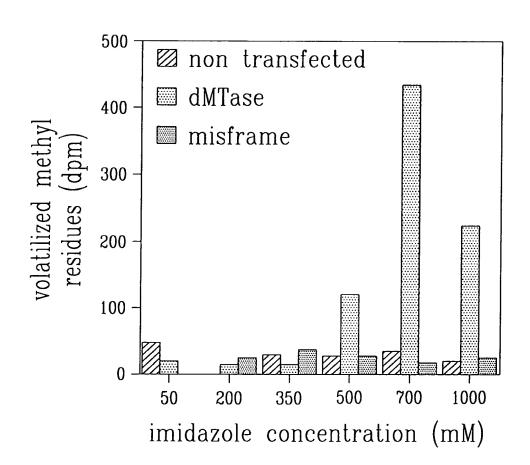






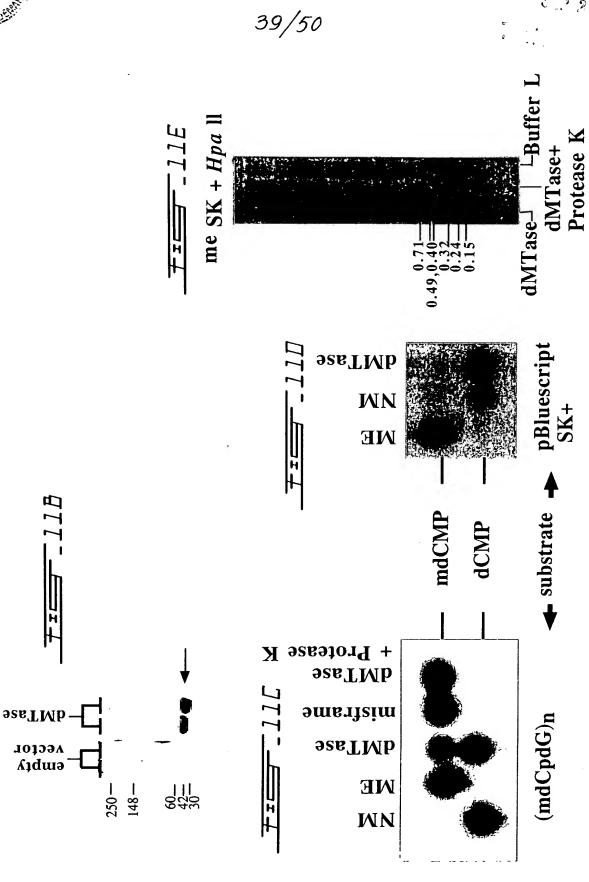


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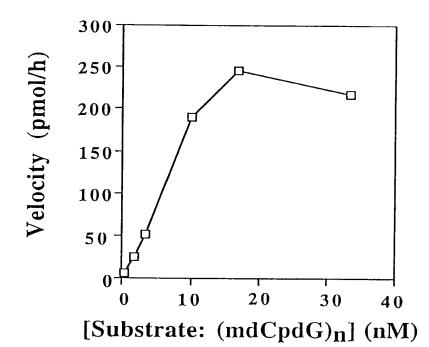


#==== 11A











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- Origin

Transient dMTase

En Estina

-Origin

A549 dMTase

- mdCMP - dCMP 100 05 01 methylated CpG DNA (ng)

-mdCMP

O O O II- dCMP

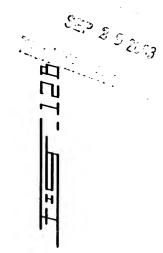
methylated CpG DNA (ng)

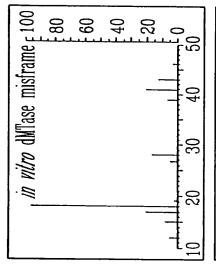
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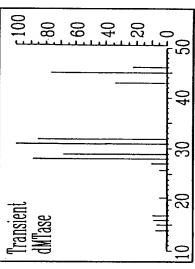
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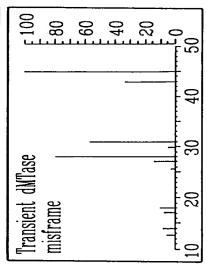
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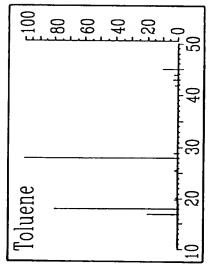


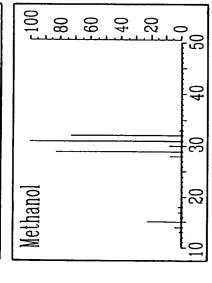


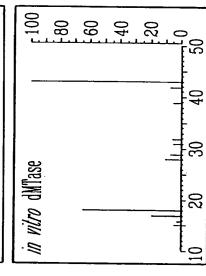




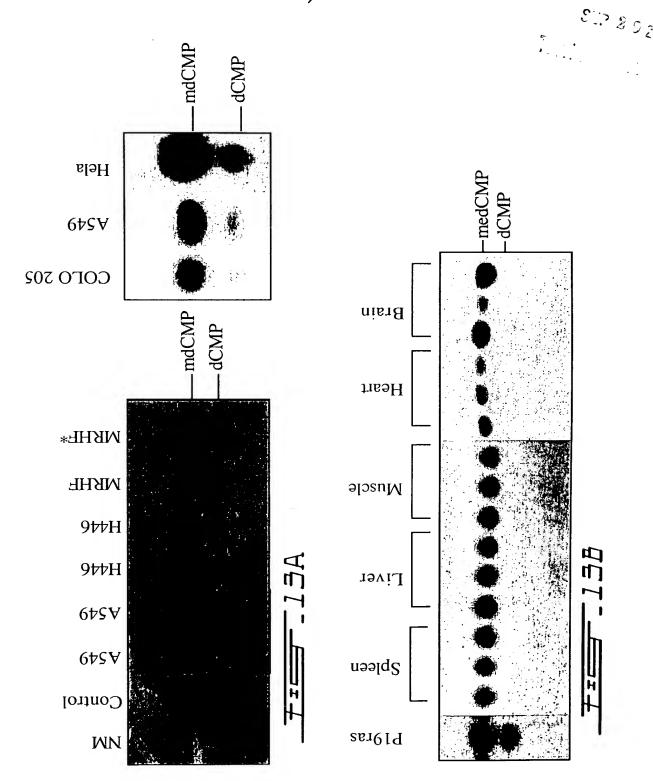




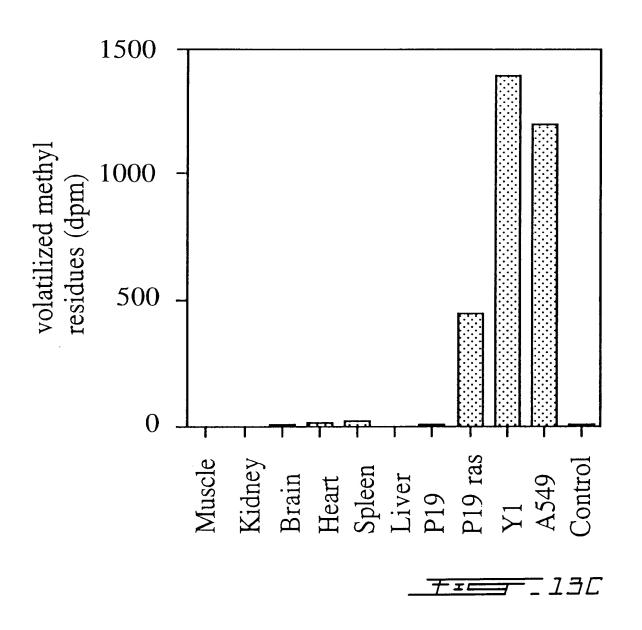




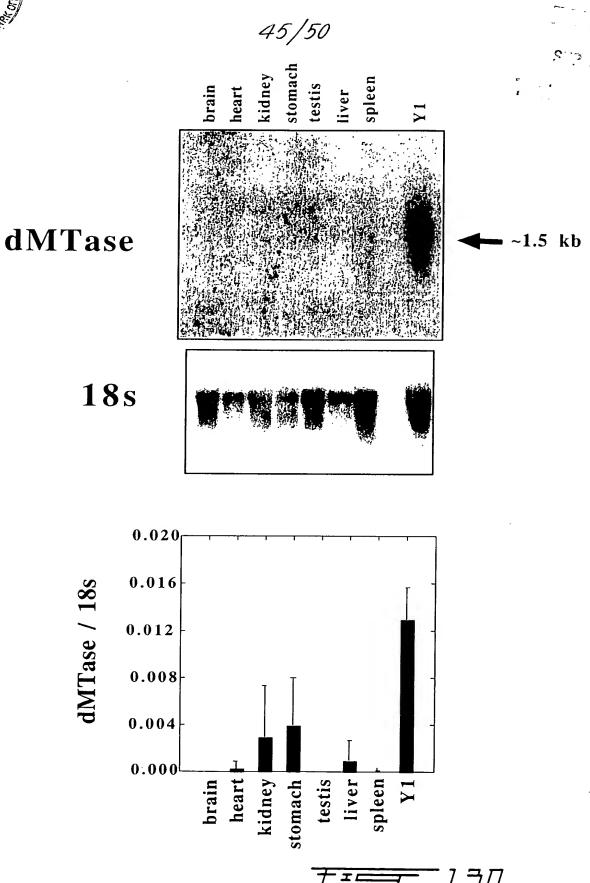


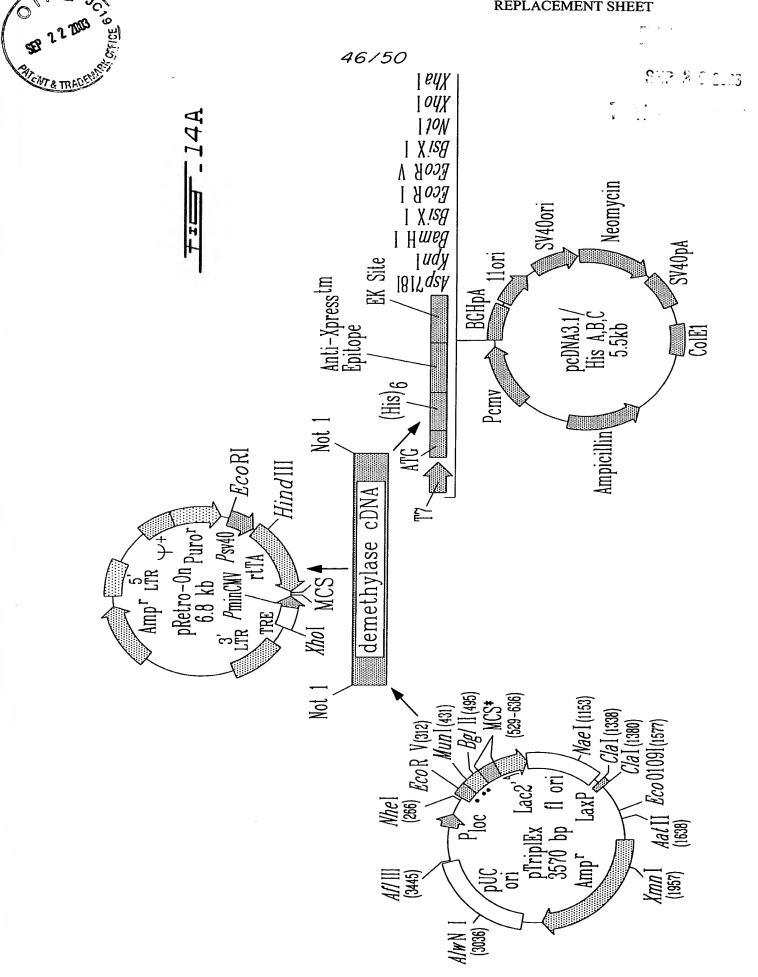




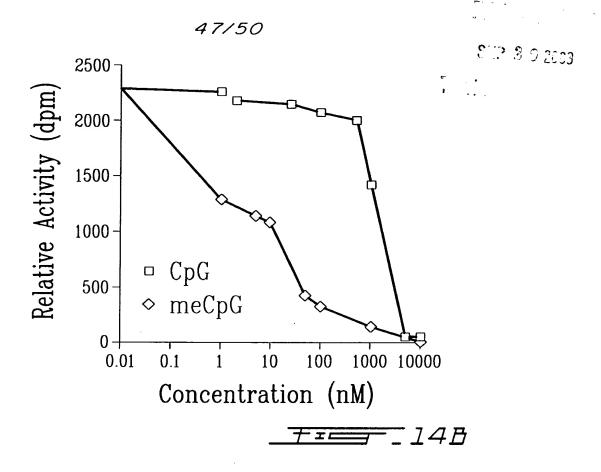


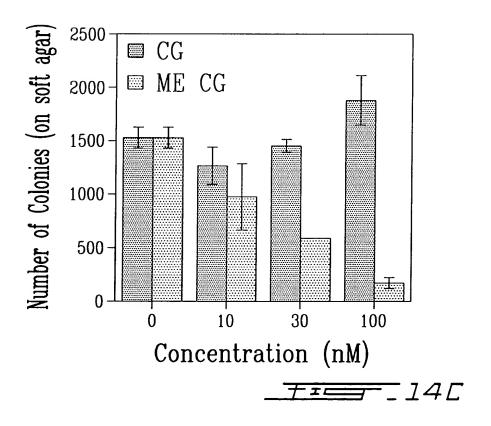






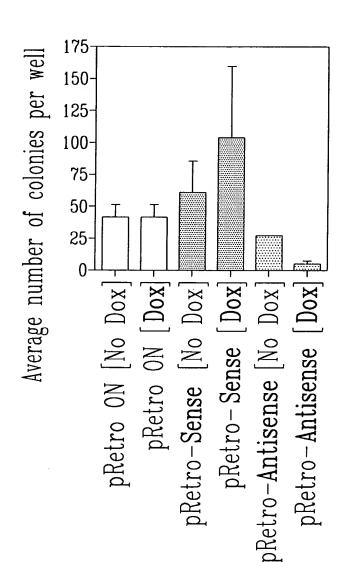


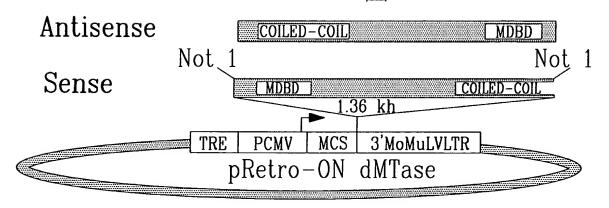




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